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Breast Cancer

Targeting the Molecular Subtypes of Triple Negative Breast Cancer: Understanding the Diversity to Progress the Field

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Key Words. Triple negative breast cancer • Molecular subtypes • Gene expression profiling • Targeted therapy

Abstract

Triple negative breast cancers (TNBCs) represent 10%–20% of primary breast cancers, and despite having greater initial sensitivity to cytotoxic chemotherapy, patients with TNBCs have higher rates of distant metastasis and a poorer prognosis compared with patients with hormone receptor positive and/or human epidermal growth factor receptor 2 positive disease. TNBC has historically been treated as a single disease entity in targeted therapy trials, but advances in gene expression profiling and other molecular diagnostic techniques over the last decade have revealed considerable biologic heterogeneity within TNBCs, including subgroups with distinct, targetable aberrations. Such molecular heterogeneity explains, in part, the disappointing performance of targeted therapeutics in unselected TNBC. Here we discuss the history of gene expression profiling in breast cancer and its application in partitioning TNBCs into subtypes that may lead to more consistent therapeutic successes in this heterogeneous disease. ***The Oncologist*** 2017;22:1086–1093

Implications For Practice: Triple negative breast cancers (TNBCs) have historically been regarded as a single entity in clinical trial design. Over the last decade, molecular characterization has revealed much heterogeneity in TNBCs, explaining in part the lackluster performance of targeted therapeutics in TNBCs as a group. In this article, we review the history of the molecular classification of breast cancer based on gene expression profiling and discuss its role in TNBCs.

Introduction

Triple negative breast cancers (TNBCs) account for approximately 10%–20% of primary breast cancers [1–4] and are characterized by a lack of expression of the estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). Compared with their ER/PR positive and/or HER2 positive counterparts, TNBCs are, in general, larger, of higher grade, and more likely to be node positive [5]. Although more likely to respond to neoadjuvant chemotherapy, chemotherapy-insensitive TNBC is associated with a poorer prognosis compared with other breast cancer subtypes for which targeted therapy either enhances chemotherapy effect or treats chemo-insensitive disease [5–7].

Despite a plethora of valid basic science research supporting the use of targeted therapy in TNBC and numerous clinical trials of active targeted agents for the treatment of TNBC, not a single targeted therapy has been U.S. Food and Drug Administration (FDA)-approved for the treatment of this particularly aggressive form of breast cancer. In addition to the known challenges for developing targeted agents in cancer such as clonal selection of resistant cells or activation of "escape" pathways, drug development in TNBC has further complexities that are inherent to clinical trial design rather than disease resistance. The lack of consistent success in the treatment of TNBC has been attributed in part to the underlying molecular heterogeneity of TNBCs.

Recent advances in gene expression profiling have identified subgroups of TNBC with distinct molecular features that, if appropriately selected, may be more responsive to targeted therapy with existing FDA-approved drugs, leading to rapid improvement of outcomes in this high-risk breast cancer population [8–11]. Here we review recent attempts to classify TNBCs into various subtypes and their implications for the development of targeted therapies.

History of Gene Expression Profiling in TNBC

Historically, breast cancers have been divided into subtypes based on differential expression of ER/PR, and subsequently HER2 by immunohistochemistry (IHC). The emergence of more sophisticated techniques in molecular biology in the late 1990s led to the use of in situ hybridization as an adjunctive test of

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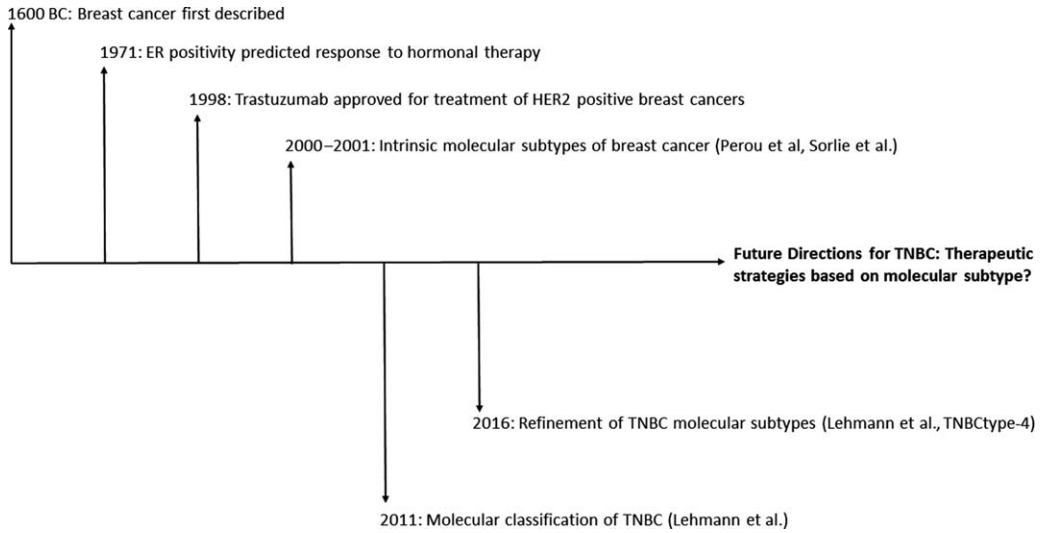

Figure 1. Classifying breast cancer. A schematic diagram summarizing the historical approaches to classifying breast cancer.
Abbreviations: ER, estrogen receptor; HER2, human epidermal growth receptor 2; TNBC, triple negative breast cancer.

HER2 status by quantifying gene amplification. More recently, gene expression profiling has been used to identify subtypes in breast cancer with a focus on TNBC where no clinically actionable subtypes currently exist (Fig. 1).

One of the earliest signs of heterogeneity of gene expression profiles in breast cancer was described by Perou et al. [12] when they, through the use of cDNA microarrays, subclassified breast cancer into four broad clusters based on their gene expression profiles: (a) luminal/ER gene cluster, (b) *ERBB2* overexpression cluster (ERBB2+), (c) basal epithelial associated cluster, and (d) normal-breast-like cluster.

The luminal subtype displayed patterns of gene expression reminiscent of luminal epithelial cells, including cytokeratins 8/18, ER, and other genes associated with ER activation [13]. A subsequent analysis then revealed that the luminal subtype could be further divided into at least two different subtypes based on differential expression of luminal specific genes, including the ER cluster of genes, giving rise to the terms "luminal A" and "luminal B" [14]. Consequently, these five distinct molecular subtypes of breast cancer (luminal A, luminal B, ERBB2+, basal-like, normal breast-like) became what is now commonly known as the "intrinsic" molecular subtypes of breast cancer (Fig. 1).

The *ERBB2* overexpression cluster had a characteristic overexpression of genes located in the same region of chromosome 17 as the *ERBB2* locus [12]. Although clinically HER2-positive tumors (by IHC or fluorescent in situ hybridization) are sometimes referred to interchangeably with tumors from the *ERBB2* overexpression cluster, this relationship is not exact because clinically HER2-positive tumors that are also hormone receptor positive may have gene expression profiles that more closely resemble those in the luminal subtypes [14–16].

In contrast to the luminal and ERBB2+ subtypes, the basal epithelial associated cluster comprised tumors that had expression profiles similar to basal epithelial cells [12], characterized by the lack of expression of ER and HER2, overexpression of basal cytokeratins, and proliferation-related genes [12, 15]. Although breast cancers with a basal-like gene expression profile tend to be TNBCs [17, 18], the converse is not true, as there is still considerable heterogeneity in gene expression profiles within TNBCs [19, 20].

Finally, the normal-breast-like cluster was characterized by high expression of basal epithelial and adipocyte-associated genes and low expression of luminal epithelial-associated genes. In the study by Perou et al., a single fibroadenoma, three normal breast specimens, and several tumor samples were assigned to this cluster [12].

Later, Prat et al. [21] described the phenotypic and molecular characteristics of yet another subtype—the claudin-low tumors, which are characterized by low expression of tight junction proteins (claudin 3, 4, and 7, as well as E-cadherin). 61%–71% of claudin-low tumors are TNBCs with a relatively high frequency of metaplastic or medullary differentiation. These tumors also demonstrated a cell surface marker expression pattern similar to mammary stem cells and breast tumor-initiating cells.

The identification of these "intrinsic" subtypes of breast cancer led to several studies evaluating the impact of specific subtypes on prognosis [14, 16, 22] and response to chemotherapy [23], in which the luminal subtypes were found to have a more indolent course and the basal subtype, while associated with poorer prognosis, had higher response rates to chemotherapy. Parker et al. developed a standardized method of identifying the "intrinsic" subtypes of breast cancer by applying a Prediction Analysis of Microarray (PAM) algorithm to a 50 gene set, commonly known as PAM50 [24], which has been used in a variety of investigational settings. A recent multicenter phase II trial of platinum monotherapy in metastatic TNBC showed a trend toward increased objective response rate (ORR) in basal versus nonbasal TNBC as defined by PAM50, but this was not statistically significant [25]. In the neoadjuvant setting, retrospective molecular analysis of pretreatment tumor samples obtained during the CALGB 40603 study showed that the benefit of adding carboplatin was consistent across all PAM50 subtypes, including nonbasal TNBCs [26, 27]. Therefore, at this point in time, the commercially available PAM50 assay has limited utility in guiding therapy for most cases of TNBC.

In an effort to better understand the heterogeneity of TNBCs, Lehmann et al. [9] analyzed gene expression profiles of

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Table 1. Distribution of molecular subtypes in triple negative breast cancer

TNBC molecular subtype[b]	Intrinsic molecular subtype[a]						
	Basal-like	ERBB2+	Luminal A	Luminal B	Normal breast-like	Unclassified	Total
BL1	85	1	1	3	5	6	101
BL2	20	8	9	9	14	18	78
IM	61	9	11	6	5	24	116
LAR	0	7	43	6	2	4	62
M	54	1	6	8	26	21	116
MSL	1	0	19	3	21	7	51
Unstable	37	0	0	7	0	5	49
Unclassified	3	2	1	0	2	5	13
Total	261	28	90	42	75	90	586[c]

[a]Intrinsic molecular subtype as defined by [14].
[b]TNBC molecular subtype as defined by [9].
[c]Of the 587 TNBC samples evaluated by [9], information on the corresponding intrinsic subtype for 586 samples were available in the supplementary material.
Abbreviations: BL1, basal-like 1; BL2, basal-like 2; ERBB2+, *ERBB2* overexpression cluster, IM, immunomodulatory; LAR, luminal androgen receptor; M, mesenchymal; MSL, mesenchymal stem-like; TNBC, triple negative breast cancer.

587 TNBCs across 21 breast cancer data sets through cluster analysis based on differential expression of a set of 2,188 genes and identified six stable TNBC subtypes, including two basal-like (BL1 and BL2), an immunomodulatory (IM), a mesenchymal (M), a mesenchymal stem-like (MSL), and a luminal androgen receptor (LAR) subtype. Later, this group used histopathological quantification and laser capture microdissection to demonstrate that transcripts used to define the IM and MSL subtypes were from tumor-infiltrating lymphocytes and peritumoral stromal cells, respectively [28]. This group also characterized TNBC cell lines based upon these signatures and demonstrated that BL1 and BL2 subtypes preferentially responded to cisplatin, whereas the M and MSL subtypes responded to inhibition of the phosphoinositide 3-kinase (PI3K)/mTOR and Abl/Src pathways, and the LAR subtype was exquisitely sensitive to AR antagonism, suggesting that gene expression analysis could help match patients with TNBCs to appropriate targeted therapies.

Subsequently, Burstein et al. analyzed RNA and DNA profiling from 198 TNBC tumors and, like Lehmann et al., identified distinct LAR, mesenchymal, and basal subtypes; however, they proposed the segregation of the basal-like subtype into basal-like immune suppressed (BLIS) and basal-like immune activated (BLIA) subtypes [29]. This alternate classification was proposed in part because the BL1 and BL2 subtypes proposed by Lehmann et al. were not readily distinguishable using hierarchical clustering of public TNBC data sets. The proposed BLIS subtype exhibited downregulation of B cell, T cell, and natural killer cell immune-regulating pathways and cytokine pathways and had the worst prognosis. In contrast, the BLIA subtype had an upregulation of immune-associated pathways and had the best prognosis.

Targeting the Subtypes of TNBC for Therapeutic Benefit

Basal-Like Triple Negative Breast Cancers

Early studies of gene expression profiling in breast cancer demonstrated that approximately 60%–72% of TNBCs and 80%–90% of breast cancers in patients with germline *BRCA1* mutations had a basal-like pattern of gene expression [19, 25, 30, 31]. This phenotypic similarity led to the hypothesis that defects in homologous recombination-mediated DNA repair pathways were central to the development of basal-like TNBCs, suggesting that agents that exploited this deficiency could potentially be successful in the clinic [32]. A single arm phase II study evaluating neoadjuvant single-agent cisplatin in patients with TNBC reported a pathologic complete response (pCR) rate of only 22% despite the fact that all patients on this study had a basal-like gene expression profile [33]. In addition, because it was found that the epidermal growth factor receptor (EGFR) was overexpressed in basal-like breast cancer [16, 18, 34], anti-EGFR therapy was thought to hold promise for TNBC and, in particular, the basal-like subtype. In a randomized phase II study of cetuximab in combination with carboplatin in metastatic TNBC, response rates to the combination was reported to be 17%. However, in the subset of patients who had basal-like tumors, the response rate to the combination was 8% [35]. Together, these observations suggest a greater degree of molecular heterogeneity in the basal-like subtype of TNBCs than was initially appreciated. Notably, in the study by Lehmann et al. [9], TNBC samples that would have been classified as basal-like based on the intrinsic molecular classification system proposed by Sorlie et al. [14, 22] were found not only in the BL1 and BL2 subtypes, but also in the IM, M, MSL and unstable subtypes, providing further evidence of heterogeneity within the original basal-like subtype (Table 1). BL1 TNBCs make up 18% of TNBCs and are characterized by high levels of expression of genes involved in the cell cycle and DNA-damage repair pathways. In contrast, BL2 TNBCs, which represent 13% of TNBCs, demonstrate upregulation of growth factor signaling pathways, including the epidermal growth factor (EGF), nerve growth factor (NGF), and MET pathways, as well as genes involved in glycolysis and gluconeogenesis. It therefore follows that BL1 TNBCs should demonstrate greater sensitivity to strategies targeting the DNA-repair pathways such as platinum-based chemotherapy and poly-ADP ribose polymerase (PARP) inhibition, whereas BL2 TNBCs should, theoretically, respond better to small molecule inhibitors of growth factor pathways. A study by Ueno et al. [36] showed that BL1 tumors had a pCR rate of 52% to neoadjuvant chemotherapy with anthracyclines

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and/or taxanes, whereas the pCR rate in BL2 tumors was 0%, providing orthogonal evidence that BL1 and BL2 are molecularly distinct entities that can be expected to respond differently to similar therapies.

Mesenchymal and Mesenchymal Stem Cell-Like Triple Negative Breast Cancers

The M and MSL TNBCs as defined by Lehmann et al. displayed upregulation of pathways involved in epithelial-to-mesenchymal transition (EMT). The M subtype is heavily enriched in pathways central to cell motility, extracellular matrix receptor interaction, and cellular differentiation. Although the MSL subtype is similarly enriched for pathways that are upregulated in the M subtype, expression of stem cell-associated genes, as well as genes involved in certain growth factor signaling pathways and angiogenesis, is uniquely associated with the MSL subtype. In addition, the MSL subtype also showed limited expression of claudins 3, 4, and 7, similar to the claudin-low subtype described by Prat et al. Mesenchymal TNBCs are enriched in epithelial-to-mesenchymal transition (EMT) and cancer stem-cell (CSC) features and contain a high rate of aberrations in the PI3K/AKT/mTOR pathway, raising the possibility of targeting this axis for the treatment of this subset [8, 9, 21, 37].

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As with all subtypes of TNBC, there is no clinical laboratory improvement amendments (CLIA)-certified diagnostic assay to identify mesenchymal TNBC, making patient selection for therapeutic trials challenging. Metaplastic breast cancer (MpBC) is a rare subtype of TNBC that can be clinically identified by light microscopy due to an admixture of epithelial and mesenchymal components within the tumor [38–44]. Approximately 10%–30% of TNBC tumors classified as mesenchymal by gene signature were found to be MpBCs based upon their morphologic features [21, 45]. Like mesenchymal TNBCs, MpBCs are often considered refractory to chemotherapy and have gene signatures that show enrichment in CSC and EMT features [8, 46–49]. MpBCs also have a high rate of aberrations in the PI3K/AKT/mTOR pathway and display high levels of angiogenesis characterized by expression of vascular endothelial growth factor and hypoxia-induced factor 1 alpha [50]. Given these features, MpBC may represent a "surrogate of response" for targeted therapy trials in mesenchymal TNBC [8]. Interestingly, a phase I study with dose expansion of liposomal doxorubicin, bevacizumab, and mTOR inhibition with temsirolimus or everolimus in patients with MpBC ($n = 52$) demonstrated an ORR of 21% with durable complete responses (CRs; 8%) and a clinical benefit rate (CBR; sum of CR, partial response [PR], and disease stability for greater than 6 months) of 40%. Of note, 74% of patients enrolled in this study had evidence of activating aberrations in the PI3K/AKT/mTOR pathway and the ORR was significantly higher in patients whose tumors had evidence of such aberrations [51].

Activation of the MET pathway has been associated with EMT and tumor progression [52], and although early results with c-MET-directed therapy have been disappointing in unselected TNBC patients with metastatic disease [53], tyrosine-protein kinase MET (c-MET)-targeted therapy could prove to be beneficial in patients who have the M or MSL subtype of TNBC. In addition, preclinical data have shown that inhibiting the transforming growth factor beta (TGF-β) receptor kinase can reverse EMT in vitro [54] and may represent a potential therapeutic opportunity. The NOTCH pathway has been implicated in the survival of stem cell-like initiating cells, and the MSL subtype may prove to be sensitive to NOTCH inhibition. A recent phase I study of the gamma secretase inhibitor, PF-03084014, in combination with docetaxel in unselected patients with advanced TNBC, reported that 16% and 44% of patients had a confirmed PR and stable disease (SD), respectively [55].

Luminal Androgen Receptor Subtype

The LAR subtype is associated with the preferential expression of genes involved in the metabolism of androgens and estrogens. The LAR subtype represents approximately 11% of TNBCs [9] and is associated with dismal response rates to cytotoxic chemotherapy [36]. Lehmann et al. also demonstrated that TNBCs belonging to the LAR subtype had significantly higher levels of AR expression assessed by IHC [9], leading to its use as a surrogate marker for the LAR subtype in clinical trials [56, 57]. In a single arm phase II trial of bicalutamide in patients with AR-positive, ER-/PR-negative metastatic breast cancer, the CBR at 24 weeks, defined as the percentage of patients who show a CR, PR, or SD at 24 weeks, was 19% and the median progression-free survival (PFS) was 12 weeks [56]. Interim results from an ongoing phase II study of enzalutamide in advanced AR-positive TNBC have been encouraging, showing a CBR at 24 weeks of 29% and a median PFS of 14 weeks. Interestingly, it was also noted that patients whose tumors demonstrated an androgen-related gene signature profile appeared to derive greater clinical benefit from enzalutamide, suggesting that the use of gene expression profiling could help identify TNBC patients who would most likely benefit from androgen receptor blockade [57].

Immunomodulatory Subtype

While the hallmark of the IM subtype as described by Lehmann et al. is a shift towards the expression of genes involved in immune signaling pathways, it is unclear if this represents the true gene expression profile of tumor cells or if this is a mere reflection of a tumor which has a significant immune infiltrate [10]. Interestingly, the BLIA subtype described by Burstein et al. overexpressed cytotoxic T lymphocyte-associated protein 4 (CTLA-4) in addition to other immune related genes and was associated with better prognosis [29]. It is conceivable that there would be considerable overlap between tumors belonging to the IM and BLIA subtypes given the similarities in their gene expression profiles. We therefore hypothesize that the IM and BLIA subtypes would prove to be responsive to immune-based therapies such as check point inhibitors and tumor vaccines. Recently published results from the phase Ib study of pembrolizumab in patients with advanced TNBC reported an overall response rate of 18.5% [58]. This study selected patients on the basis of programmed death-ligand 1 positivity and no

Table 2. Targeting the molecular subtypes of triple negative breast cancer

Subtype of triple negative breast cancer	Pathway	Drug
Basal-like 1/basal-like 2	DNA damage repair	PARP inhibitors Platinum compounds
	EGFR	Anti-EGFR monoclonal antibodies
Mesenchymal/mesenchymal stem-like	PI3K/AKT/mTOR	PI3K inhibitors mTOR inhibitors
	MET	c-MET inhibitors
	TGF-β	TGF-β receptor kinase inhibitors
	NOTCH	Gamma secretase inhibitors
Luminal androgen receptor	AR	AR antagonists
Immunomodulatory	Immune signaling	Check point inhibitors Vaccine therapy
HER2 enriched[a]	HER2	HER2 directed therapies

[a]Although the HER2 enriched subtype was not one of Lehmann's proposed subtypes, triple negative breast cancers that have low level HER2 overexpression likely represent a distinct group of tumors that may respond favorably to HER2-directed therapies.
Abbreviations: AR, androgen receptor; c-MET, tyrosine-protein kinase MET; EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; mTOR, mechanistic target of rapamycin; PARP, poly-ADP ribose polymerase; PI3K, phosphoinositide 3-kinase; TGF-β, transforming growth factor beta.

data on gene expression profiling have been reported. As pembrolizumab moves forward in clinical development for TNBC, it will be interesting to determine if patients with the IM and/or BLIA subtypes will have better responses compared with patients with other subtypes of TNBC.

HER2 Enriched Triple Negative Tumors

Although TNBCs are by definition HER2 negative, a subset of TNBCs demonstrate a gene expression profile similar to the *ERBB2* overexpression cluster as described by Perou et al. [12]. This is an important consideration as centralized testing of tumor samples from the NSABP-B31 trial [59] showed that 10% of the patients treated with trastuzumab had tumors that lacked HER2 overexpression by IHC upon central review. Interestingly, trastuzumab appeared to have clinical benefit despite the absence of HER2 overexpression in a subset analysis of this patient population. Although not statistically significant, another subset analysis from a randomized phase II trial of the HER2 peptide vaccine AE37 showed a trend toward improved disease-free survival in TNBC patients with low-level HER2 expression who received the vaccine compared with those who did not [60]. Further data are needed to fully characterize the impact of HER2-directed therapies in TNBCs, and the ongoing phase III NASBP B47 trial should provide more clarity in this area. It is also plausible that gene signatures may one day aid in identifying TNBC patients who will benefit from HER2-directed therapies.

Identification of Subtypes Based on Somatic and Germline Mutations

With the widespread availability of high throughput sequencing, attempts to characterize TNBCs based on their somatic mutational landscape and potentially identify common targetable driver events have yielded some interesting observations. Not surprisingly, whole exome sequencing of genomic DNA from unselected TNBC cases at diagnosis demonstrated a wide variation in genomic evolution with some cases demonstrating low-clonality (fewer somatic mutations at higher allelic frequencies) and others showing evidence of more extensive clonal evolution (multiple somatic mutations at lower allelic frequencies) [61]. Although the relationship is not exact, basal-like TNBCs tend to have more extensive clonal evolution compared with non-basal-like TNBCs. *TP53* is the most commonly mutated gene in TNBC (60%–75%), but its role in predicting sensitivity to chemotherapy and long term outcomes in TNBC is controversial [25, 62]. The *PIK3CA* gene is another commonly mutated gene in TNBC (9%) [61], and efforts are underway to further define the role of PI3K inhibition in TNBC. While somatic mutations in *TP53*, *PIK3CA* and *PTEN* have been identified as clonally dominant genetic alterations in a substantial proportion of tumors, their clonal frequencies in a subset of tumors were inconsistent with founder status, suggesting variation in founder events resulting in carcinogenesis [61]. Despite current limitations, information derived from somatic mutation analysis of tumor specimens could complement the use of gene expression profiling in clinical practice. For example, Lehmann et al. reported that TNBC cell lines resembling the LAR and M/MSL subtypes demonstrated significant sensitivity to PI3K inhibition, which correlated with the presence of mutations in *PIK3CA* [9]. Currently ongoing trials are exploring the combination of AR blockade with PI3K inhibition, which may prove to be synergistic in patients with the LAR subtype of TNBC.

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In recent years, emerging data from clinical trials have suggested that the presence of germline mutations in *BRCA1* and *BRCA2* could potentially help inform treatment decisions. In the TNT trial, patients with metastatic or recurrent locally advanced breast cancer that was either triple negative or associated with germline mutations in *BRCA1* or *BRCA2* were randomized to receive either carboplatin or docetaxel. In the

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unselected TNBC population, there was no evidence to support the superiority of either agent. However, in patients with germline mutations in *BRCA1* or *BRCA2*, carboplatin was associated with a higher response rate [63]. Of note, 55% (16/29) of patients with germline mutations in *BRCA1* or *BRCA2* in this study had TNBC. More recently, a press release reported that the OlympiAD trial, which randomized patients harboring germline mutations of *BRCA1* or *BRCA2* with HER2-negative metastatic breast cancer to receive either olaparib or physician's choice chemotherapy, demonstrated that patients receiving olaparib benefitted from a statistically significant and clinically meaningful improvement in PFS. We anticipate that full details of the results will be released soon, further enhancing our ability to make therapeutic decisions for TNBC patients who harbor germline *BRCA1* or *BRCA2* mutations.

Molecular Classification and Potential Treatment Strategies

It is hoped that the identification of TNBC subtypes will lead to therapeutic advances in the treatment of TNBC, as each subtype has molecular aberrations potentially targetable with existing FDA-approved drugs or agents currently under development (Table 2). For example, identification of the BL1 subset and its dependence on the DNA repair pathway suggests that future studies targeting the BL1 subset could exploit this dependence through the use of DNA-damaging agents such as platinum compounds and/or PARP inhibitors to improve therapeutic efficacy compared with treatment of unselected TNBC patients. Also, data from early phase trials are suggesting that targeted therapy such as androgen antagonists for AR-positive TNBC [56, 57] or mTOR inhibition in mesenchymal/metaplastic TNBC [51] may be viable options for the treatment of specific subsets of TNBC. Lastly, several treatment strategies that could potentially prove to be effective in treating the additional TNBC subtypes are currently under investigation, including the use of drugs targeting the EMT pathway and cancer stem cells, as well as immune-directed therapies.

The Way Forward

At the present moment, classifying TNBCs into molecular subtypes based on gene expression profiling remains experimental. The difficulty in applying large-scale gene expression data to clinical practice is in part due to the large number of genes involved, which invariably results in overfitting of data due to the inclusion of genes that have little or no impact on outcome, resulting in a less than ideal performance when applied in real life. Building on earlier work by Lehmann et al., Ring et al. developed a new classification model based on 101 genes using the same gene expression data sets [64]. There was considerable agreement between the two models, and a commercial assay is being developed based on this algorithm with additional studies being planned to compare the performance of both models as predictors of prognosis and response to therapy [64].

However, creating a clinically relevant classification system for TNBC is only the first step. As our understanding of the underlying biology improves, it is highly conceivable that multiple rare and inherently different subtypes of TNBC will be identified in the future and the only way to design adequately powered clinical trials for each distinct subtype would be through greater interinstitutional collaboration.

Conclusion

The limited success of targeted therapeutics in TNBCs can be attributed in part to molecular heterogeneity within the "catch all" diagnosis of TNBC. Until recently, most clinical trials of TNBCs have enrolled unselected populations of patients with TNBC, which results in a dilution of drug effect. Although gene expression profiling has provided much insight into the underlying biology and heterogeneity of TNBCs, subtype "calls" are very much affected by the bioinformatic methods used [10] and there is currently no consensus on the optimal way of stratifying TNBCs. In addition, the use of large numbers of genes in predictive model development has often times led to overfitting, limiting reproducibility in the real world. Although multiple methods of partitioning TNBCs based on gene expression profiles have been proposed, similarities exist between the subtypes defined by each method, suggesting that we may soon adopt a uniform method of classifying TNBCs. It is imperative to continue incorporating gene expression profiling into clinical trials of targeted therapies in TNBCs, as the data generated will allow us to retrospectively match response with patterns of gene expression, thereby helping to inform the design of future prospective studies.

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References

1. Morris GJ, Naidu S, Topham AK et al. Differences in breast carcinoma characteristics in newly diagnosed African-American and Caucasian patients: A single-institution compilation compared with the National Cancer Institute's Surveillance, Epidemiology, and End Results database. Cancer 2007;110: 876–884.

2. Onitilo AA, Engel JM, Greenlee RT et al. Breast cancer subtypes based on ER/PR and Her2 expression: Comparison of clinicopathologic features and survival. Clin Med Res 2009;7:4–13.

3. Bauer KR, Brown M, Cress RD et al. Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype: A population-based study from the California cancer registry. Cancer 2007;109: 1721–1728.

4. Thike AA, Cheok PY, Jara-Lazaro AR et al. Triple-negative breast cancer: Clinicopathological characteristics and relationship with basal-like breast cancer. Mod Pathol 2010;23:123–133.

5. Dent R, Trudeau M, Pritchard KI et al. Triple-negative breast cancer: Clinical features and patterns of recurrence. Clin Cancer Res 2007;13: 4429–4434.

6. Carey LA, Dees EC, Sawyer L et al. The triple negative paradox: Primary tumor chemosensitivity of breast cancer subtypes. Clin Cancer Res 2007;13: 2329–2334.

7. Haffty BG, Yang Q, Reiss M et al. Locoregional relapse and distant metastasis in conservatively

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managed triple negative early-stage breast cancer. J Clin Oncol 2006;24:5652–5657.

8. Hennessy BT, Gonzalez-Angulo AM, Stemke-Hale K et al. Characterization of a naturally occurring breast cancer subset enriched in epithelial-to-mesenchymal transition and stem cell characteristics. Cancer Res 2009;69:4116–4124.

9. Lehmann BD, Bauer JA, Chen X et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest 2011;121:2750–2767.

10. Lehmann BD, Pietenpol JA. Identification and use of biomarkers in treatment strategies for triple-negative breast cancer subtypes. J Pathol 2014;232: 142–150.

11. Prat A, Adamo B, Cheang MC et al. Molecular characterization of basal-like and non-basal-like triple-negative breast cancer. ***The Oncologist*** 2013;18: 123–133.

12. Perou CM, Sørlie T, Eisen MB et al. Molecular portraits of human breast tumours. Nature 2000; 406:747–752.

13. Brenton JD, Carey LA, Ahmed AA et al. Molecular classification and molecular forecasting of breast cancer: Ready for clinical application? J Clin Oncol 2005;23:7350–7360.

14. Sørlie T, Perou CM, Tibshirani R et al. Gene expression patterns of breast carcinomas distinguish tumor subclasses with clinical implications. Proc Natl Acad Sci USA 2001;98:10869–10874.

15. Sotiriou C, Neo SY, McShane LM et al. Breast cancer classification and prognosis based on gene expression profiles from a population-based study. Proc Natl Acad Sci USA 2003;100:10393–10398.

16. Sorlie T, Tibshirani R, Parker J et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. Proc Natl Acad Sci USA 2003;100:8418–8423.

17. Livasy CA, Karaca G, Nanda R et al. Phenotypic evaluation of the basal-like subtype of invasive breast carcinoma. Mod Pathol 2006;19:264–271.

18. Nielsen TO, Hsu FD, Jensen K et al. Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. Clin Cancer Res 2004;10:5367–5374.

19. Bertucci F, Finetti P, Cervera N et al. How basal are triple-negative breast cancers? Int J Cancer 2008; 123:236–240.

20. Prat A, Perou CM. Deconstructing the molecular portraits of breast cancer. Mol Oncol 2011;5:5–23.

21. Prat A, Parker JS, Karginova O et al. Phenotypic and molecular characterization of the claudin-low intrinsic subtype of breast cancer. Breast Cancer Res 2010;12:R68.

22. Hu Z, Fan C, Oh DS et al. The molecular portraits of breast tumors are conserved across microarray platforms. BMC Genomics 2006;7:96.

23. Prat A, Fan C, Fernández A et al. Response and survival of breast cancer intrinsic subtypes following multi-agent neoadjuvant chemotherapy. BMC Med 2015;13:303.

24. Parker JS, Mullins M, Cheang MC et al. Supervised risk predictor of breast cancer based on intrinsic subtypes. J Clin Oncol 2009;27:1160–1167.

25. Isakoff SJ, Mayer EL, He L et al. TBCRC009: A multicenter phase II clinical trial of platinum monotherapy with biomarker assessment in metastatic triple-negative breast cancer. J Clin Oncol 2015;33: 1902–1909.

26. Sikov WM, Berry DA, Perou CM et al. Impact of the addition of carboplatin and/or bevacizumab to neoadjuvant once-per-week paclitaxel followed by dose-dense doxorubicin and cyclophosphamide on pathologic complete response rates in stage II to III triple-negative breast cancer: CALGB 40603 (Alliance). J Clin Oncol 2015;33:13–21.

27. Sikov WM, Barry WT, Hoadley KA et al. Impact of intrinsic subtype by PAM50 and other gene signatures on pathologic complete response (pCR) rates in triple-negative breast cancer (TNBC) after neoadjuvant chemotherapy (NACT) +/- carboplatin (Cb) or bevacizumab (Bev): CALGB 40603/150709 (Alliance). San Antonio Breast Cancer Symposium; 9–13 December 2014:S4-05a.

28. Lehmann BD, Jovanović B, Chen X et al. Refinement of triple-negative breast cancer molecular subtypes: Implications for neoadjuvant chemotherapy selection. PLoS One 2016;11:e0157368.

29. Burstein MD, Tsimelzon A, Poage GM et al. Comprehensive genomic analysis identifies novel subtypes and targets of triple-negative breast cancer. Clin Cancer Res 2015;21:1688–1698.

30. Foulkes WD, Stefansson IM, Chappuis PO et al. Germline BRCA1 mutations and a basal epithelial phenotype in breast cancer. J Natl Cancer Inst 2003; 95:1482–1485.

31. Lakhani SR, Reis-Filho JS, Fulford L et al. Prediction of BRCA1 status in patients with breast cancer using estrogen receptor and basal phenotype. Clin Cancer Res 2005;11:5175–5180.

32. Turner NC, Reis-Filho JS, Russell AM et al. BRCA1 dysfunction in sporadic basal-like breast cancer. Oncogene 2007;26:2126–2132.

33. Silver DP, Richardson AL, Eklund AC et al. Efficacy of neoadjuvant cisplatin in triple-negative breast cancer. J Clin Oncol 2010;28:1145–1153.

34. Hoadley KA, Weigman VJ, Fan C et al. EGFR associated expression profiles vary with breast tumor subtype. BMC Genomics 2007;8:258.

35. Carey LA, Rugo HS, Marcom PK et al. Tbcrc 001: Randomized phase ii study of cetuximab in combination with carboplatin in stage iv triple-negative breast cancer. J Clin Oncol 2012;30: 2615–2623.

36. Masuda H, Baggerly KA, Wang Y et al. Differential response to neoadjuvant chemotherapy among 7 triple-negative breast cancer molecular subtypes. Clin Cancer Res 2013;19:5533–5540.

37. Yu KD, Zhu R, Zhan M et al. Identification of prognosis-relevant subgroups in patients with chemoresistant triple-negative breast cancer. Clin Cancer Res 2013;19:2723–2733.

38. Abouharb S, Moulder S. Metaplastic breast cancer: Clinical overview and molecular aberrations for potential targeted therapy. Curr Oncol Rep 2015; 17:431.

39. Tse GM, Tan PH, Putti TC et al. Metaplastic carcinoma of the breast: A clinicopathological review. J Clin Pathol 2006;59:1079–1083.

40. Wargotz ES, Norris HJ. Metaplastic carcinomas of the breast. I. Matrix-producing carcinoma. Hum Pathol 1989;20:628–635.

41. Wargotz ES, Deos PH, Norris HJ. Metaplastic carcinomas of the breast. II. Spindle cell carcinoma. Hum Pathol 1989;20:732–740.

42. Wargotz ES, Norris HJ. Metaplastic carcinomas of the breast. III. Carcinosarcoma. Cancer 1989;64: 1490–1499.

43. Wargotz ES, Norris HJ. Metaplastic carcinomas of the breast. IV. Squamous cell carcinoma of ductal origin. Cancer 1990;65:272–276.

44. Wargotz ES, Norris HJ. Metaplastic carcinomas of the breast: V. Metaplastic carcinoma with osteoclastic giant cells. Hum Pathol 1990;21: 1142–1150.

45. Gerhard R, Ricardo S, Albergaria A et al. Immunohistochemical features of claudin-low intrinsic subtype in metaplastic breast carcinomas. Breast 2012;21:354–360.

46. Hennessy BT, Giordano S, Broglio K et al. Biphasic metaplastic sarcomatoid carcinoma of the breast. Ann Oncol 2006;17:605–613.

47. Jung SY, Kim HY, Nam BH et al. Worse prognosis of metaplastic breast cancer patients than other patients with triple-negative breast cancer. Breast Cancer Res Treat 2010;120:627–637.

48. Luini A, Aguilar M, Gatti G et al. Metaplastic carcinoma of the breast, an unusual disease with worse prognosis: The experience of the european institute of oncology and review of the literature. Breast Cancer Res Treat 2007;101:349–353.

49. Rayson D, Adjei AA, Suman VJ et al. Metaplastic breast cancer: Prognosis and response to systemic therapy. Ann Oncol 1999;10:413–419.

50. Kochhar R, Howard EM, Umbreit JN et al. Metaplastic breast carcinoma with squamous differentiation: Molecular and clinical analysis of six cases. Breast J 2005;11:367–369.

51. Basho RK, Gilcrease M, Murthy RK et al. Targeting the PI3K/AKT/mTOR pathway for the treatment of mesenchymal triple-negative breast cancer: Evidence from a phase 1 trial of mTOR inhibition in combination with liposomal doxorubicin and bevacizumab. JAMA Oncol 2017;3:509–515.

52. Thiery JP. Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer 2002;2:442–454.

53. Diéras V, Campone M, Yardley DA et al. Randomized, phase II, placebo-controlled trial of onartuzumab and/or bevacizumab in combination with weekly paclitaxel in patients with metastatic triple-negative breast cancer. Ann Oncol 2015;26: 1904–1910.

54. Shipitsin M, Campbell LL, Argani P et al. Molecular definition of breast tumor heterogeneity. Cancer Cell 2007;11:259–273.

55. Locatelli MA, Aftimos P, Dees EC et al. Phase I study of the gamma secretase inhibitor PF-03084014 in combination with docetaxel in patients with advanced triple-negative breast cancer. Oncotarget 2017;8:2320–2328.

56. Gucalp A, Tolaney S, Isakoff SJ et al. Phase II trial of bicalutamide in patients with androgen receptor-positive, estrogen receptor-negative metastatic breast cancer. Clin Cancer Res 2013;19:5505–5512.

57. Traina TA MK, Yardley DA, O'Shaughnessy J. Results from a phase 2 study of enzalutamide (ENZA), an androgen receptor (AR) inhibitor, in advanced AR+ triple-negative breast cancer (TNBC). J Clin Oncol 2015;33:1003a.

58. Nanda R, Chow LQ, Dees EC et al. Pembrolizumab in patients with advanced triple-negative breast cancer: Phase Ib KEYNOTE-012 study. J Clin Oncol 2016;34:2460–2467.

59. Paik S, Kim C, Wolmark N. HER2 status and benefit from adjuvant trastuzumab in breast cancer. N Engl J Med 2008;358:1409–1411.

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60. Mittendorf EA, Ardavanis A, Symanowski J et al. Primary analysis of a prospective, randomized, single-blinded phase II trial evaluating the HER2 peptide AE37 vaccine in breast cancer patients to prevent recurrence. Ann Oncol 2016;27:1241–1248.

61. Shah SP, Roth A, Goya R et al. The clonal and mutational evolution spectrum of primary triple-negative breast cancers. Nature 2012;486:395–399.

62. Darb-Esfahani S, Denkert C, Stenzinger A et al. Role of TP53 mutations in triple negative and HER2-positive breast cancer treated with neoadjuvant anthracycline/taxane-based chemotherapy. Oncotarget 2016;7:67686–67698.

63. Tutt A EP, Kilburn L et al. The TNT trial: A randomized phase III trial of carboplatin (C) compared with docetaxel (D) for patients with metastatic or recurrent locally advanced triple negative or *BRCA1/2* breast cancer (CRUK/07/012). San Antonio Breast Cancer Symposium; 9–13 December 2014: S3-01a.

64. Ring BZ, Hout DR, Morris SW et al. Generation of an algorithm based on minimal gene sets to clinically subtype triple negative breast cancer patients. BMC Cancer 2016;16:143.

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For Further Reading:

Maggie C.U. Cheang, Miguel Martin, Torsten O. Nielsen et al. Defining Breast Cancer Intrinsic Subtypes by Quantitative Receptor Expression. ***The Oncologist*** 2015;20:474–482; first published on April 23, 2015.

Implications for Practice:

This study pooled centrally reviewed hormone receptor (HR) and HER2 data and individual gene expression and intrinsic subtyping from three cooperative group trials. The results indicated that the optimal cut point for defining triple-negative breast cancer, if the goal is to enrich for basal-like biology, is to adopt the guideline of $<1\%$ staining. Tumors with borderline HR expression are highly biologically heterogeneous, which raises the question of whether these tumors should be considered indeterminate. A proportion of clinically defined HER2-negative tumors were defined as molecular HER2-enriched subtype; however, whether they are suitable for anti-HER2 therapy needs to be determined.